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BMCL Digest

A rapid alternative to X-ray crystallography for chiral determination: Case studies of vibrational circular dichroism (VCD) to advance drug discovery projects

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ABSTRACT

The absolute stereochemistry of chiral drugs is usually established via X-ray crystallography. However, vibrational circular dichroism (VCD) spectroscopy coupled with quantum mechanics simulations offers a rapid alternative to crystallography and is readily applied to both crystalline and non-crystalline samples. VCD is an effective complement to X-ray analysis of drug candidates, and it can be used as a high-throughput means of assessing absolute stereochemistry at all phases of the discovery process (hundreds of assignments per year). The practical implementation (or fee-for-service outsourcing) of VCD and selected case studies are illustrated with an emphasis on providing utility and impact to pharmaceutical discovery programs.

Nearly two out of three drugs reaching the commercial market are chiral, and the determination of absolute stereochemistry is a central component of their discovery and development (Fig. 1).[1] Frequently, the absolute configuration of a chiral drug is not known until relatively late in the discovery phase, and most often the structure is determined using the accepted gold standard of X-ray crystallography and/or the sample is prepared using a stereo-controlled synthetic route. However, vibrational circular dichroism (VCD) spectroscopy offers a rapid alternative to crystallography and is readily applied to both crystalline and non-crystalline samples at all phases of the discovery process. This Digest provides to a medicinal chemistry audience an overview of the essentials of VCD as an emerging technology in pharmaceutical research as well as several case studies of practical examples that have impacted drug discovery programs.

A VCD spectrum is defined as the differential absorbance of left- vs. right-circularly polarized infrared light by a chiral sample:

$$\text{VCD spectrum}: \Delta A(\nu) = A_{\text{left}}(\nu) - A_{\text{right}}$$

Like electronic (UV/visible) circular dichroism spectroscopy familiar to chemists, VCD spectra of pure enantiomeric species are identical in all respects, except that the spectral features of the two enantiomers will be opposite in sign, that is, if a band is positive for one enantiomer, it will be negative for the opposite enantiomer (Fig. 2).

Relative to CD analysis, VCD has the technical challenge of limited sensitivity, but offers the benefit of numerous, well-defined bands with which to assign the absolute configuration. Analogous

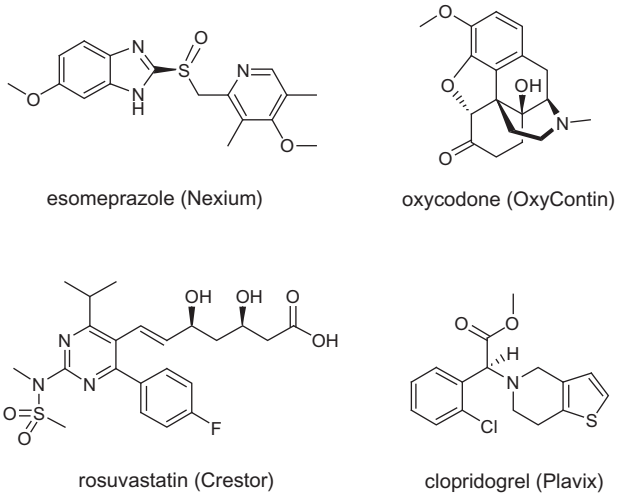

Figure 1. Examples of major marketed drugs with at least one chiral center.

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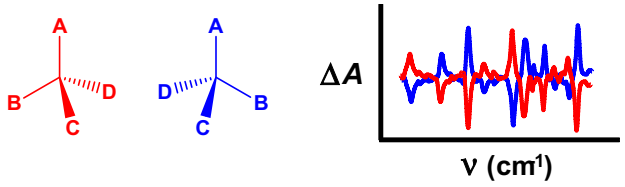


Figure 2. VCD spectra for an enantiomeric pair. The blue spectrum of one enantiomer is the opposite of the red spectrum of the other enantiomer. Because the spectrum is collected in the fingerprint region of the IR, the number of peaks (i.e., the information density) is generally greater than that of electronic CD spectra.

to the fingerprint region of classical IR spectra, VCD spectra typically provide information-dense patterns due to the differential absorbance associated with specific vibrational modes, most often those in close proximity to a chiral center.

While the raw VCD spectra can be used to differentiate two enantiomers (or more complex molecules with multiple chiral centers), the experimental spectra alone are not independently sufficient to assign absolute chirality. Absolute assignment requires comparison of the VCD spectrum to a reference spectrum for which the specific signs of bands are known to be directly linked to an established absolute chirality. While such a reference spectrum can be obtained from a compound for which the absolute chiral structure is known (i.e., either through X-ray crystal structure analysis or from a batch generated through a stereo-specific synthesis), most often no such prior compound or knowledge is available. In these cases the reference spectrum must be obtained from quantum mechanics simulations of the VCD spectrum (most often using density functional theory).[2–5]

Figure 3 summarizes the fundamental steps involved in VCD chiral assignment and also provides data for our first case study. For experimental VCD acquisition, typically ca. 2–10 mgs of material is sufficient to achieve a suitable signal. Neutral compounds (rather than salts) are desirable to avoid the need to model complex matrix and counter-ion effects. Ideally, samples are dissolved in a nonpolar solvent (e.g., $CDCl_3$) to minimize the effects of hydrogen bonding and leave the physical system with minimal perturbations such that the environment of the simulations more accurately mimics the conditions in the experimental cell. A number of more technically detailed overviews of VCD are also available in the literature[6–8] as well as a recent book specifically aimed toward organic chemists.[9]

The number of VCD assignments of absolute stereochemistry is estimated to be in the thousands.[10] These chiral assignments fall into three general categories:

1. Assignment of compounds for which chemists had no preconceived notion of the absolute stereochemistry.
2. Confirmation of the stereochemistry already established (or at least inferred or suspected) by the chemists.
3. Revelation that the absolute stereochemistry is not what the chemists anticipated.

All three scenarios have value, although the last is arguably the most intriguing.

Case study #1: neurokinin-3 antagonists and intermediates.

Neurokinin-3 (NK3) antagonists have been pursued for CNS indications including schizophrenia and pain, and also for potential utility in androgen-dependent diseases.[11–14] The NK3 program at AstraZeneca yielded AZD2624 which contains a chiral amide substituent (Fig. 4).[15] Since the complexity of the VCD simulations in-

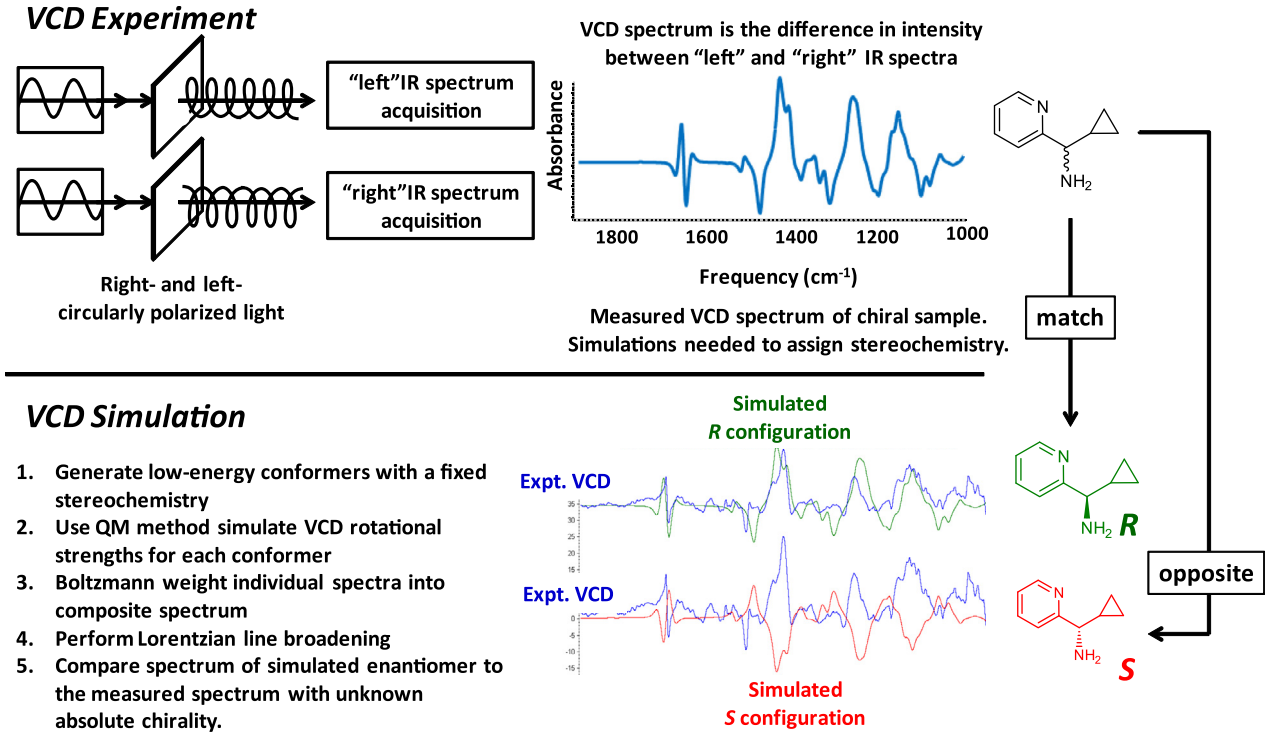


Figure 3. The essential steps in the VCD assignment of a single enantiomer sample of unknown absolute stereochemistry. The acquisition of a measured VCD spectrum requires collection of IR spectra using left- and right-circularly polarized light and generating the difference spectrum. Simulated spectra of the *R* and *S* enantiomers are constructed using quantum mechanics (QM) simulations and subsequent Boltzmann weighted co-addition of Lorentzian-broadened rotational strengths of energetically low-lying conformers. Direct comparison of the measured and simulated VCD spectra facilitates assignment, in this case a match to the simulated *R* spectrum (and opposite that of the simulated *S* spectrum).

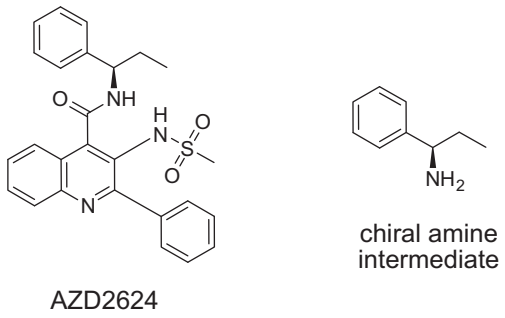


Figure 4. NK3 antagonist AZD2624. VCD assignment of the much smaller chiral amine intermediate is a practical strategy for assignment (by inference) of the final compound. This strategy requires that the stereocenter is unaffected by subsequent synthetic steps following its installation.

creases greatly with the size and flexibility of the molecule (i.e., many more conformers), a practical option is to assign the chirality of intermediates whenever possible.

While efficient, the strategy of assigning chiral intermediates relies on the integrity of the chiral center being unaltered in any subsequent chemical transformations and must be used with this qualification clearly in mind. A portion of one synthetic route for a related series of sulfoxide NK3 antagonists is shown in Figure 5.[16] The final compounds in this sub-series contain two chiral centers: the amide substituent and the sulfoxide. The amine intermediates that were separated by chiral chromatography were assigned by VCD, and the SAR of this series could then be assessed with full knowledge of the amide stereochemistry.

The chiral assignment of these small amines is straightforward and unambiguous as shown in Figure 3. Clearly, the measured cyclopropyl amine in this case can be assigned as the *S* configuration by comparison to the simulated spectra. The turnaround time for assignment of compounds of this size can be as short as a single day. For key compounds, the more complex assignment of both stereocenters was performed on each final product. While unambiguous assignments are often possible for complex compounds with multiple stereocenters, careful selection and Boltzmann weighting of the relevant conformers in the simulated spectra are essential. Quite often, the most practical and cost-effective

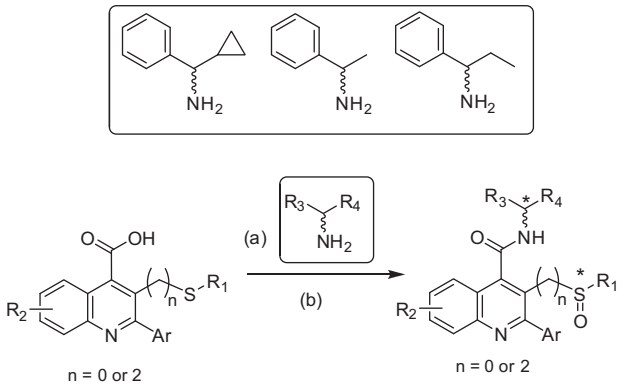


Figure 5. Example synthetic route of NK3 antagonists (see Ref. 16): (a) amine, EDCI, HOBt, DIPEA, DCM; (b) $NaIO_4$, $MeOH/H_2O$, The VCD assignment of a variety of chiral amines (e.g., cyclopropyl analog in Fig. 3) allows the selection of the desired chiral amine in step (a) and the assignment of this stereocenter in the final product. For key compounds, the absolute configuration (both the amide portion and the sulfoxide chirality) was determined for the final product by comparing measured spectra to all four possible simulated spectra (*R,R*; *S,S*; *R,S*; *S,R*) and ultimately confirmed by X-ray crystallography.

chiral assignments for a series of compounds can be realized by characterizing the smallest and least conformationally flexible intermediate that has the stereocenter installed (provided that it is assured to be retained in subsequent steps). For key compounds, assessment of the final compound is always advisable.

Case study #2: re-assignment of chirality of a histamine-3 tool compound.

Since the discovery of the histamine-3 (H_3) receptor in 1983[17] and its subsequent cloning in 1999,[18] H_3 antagonists and inverse agonists have been sought for indications ranging from cognitive deficits in Alzheimer's disease to Tourette syndrome, with a number of compounds currently in clinical trials.[19,20] One of the first tool compounds used widely in H_3 pharmaceutical research is cipralisant, aka Gliatech's GT-2331 (Fig. 6).[21] The structure of cipralisant was published as the dextrorotatory (+) enantiomer with (1*R*,2*R*) absolute stereochemistry assigned by X-ray crystallography based on a sultam derivative.[22,23] Later, Liu et al. at Abbott prepared cipralisant and described a large scale synthesis of this important reference compound.[24] Additionally, their X-ray crystallography of the sultam intermediate as well as the L-tartrate and D-tartrate salts of cipralisant itself suggest that while the more biologically active enantiomer is the dextrorotatory (+) enantiomer, its true absolute stereochemistry is instead (1*S*,2*S*).

Minick et al. at GSK played the additional role of arbiter using VCD as an independent method for determining the absolute configuration of cipralisant.[25] Their thorough investigation of the imidazole tautomeric states and hydrogen bonded dimers provided conclusive and independent evidence for cipralisant having (1*S*,2*S*) absolute stereochemistry. It represents a classic case of working through the additional complexities of simulating key elements of the physical system in solution (tautomeric state, interactions in solution, etc.) that are sometimes required to achieve a reliable match between simulated and measured spectra, that is, it is not always a turn-key technique. It also reminds us that while X-ray crystallography remains the gold standard for chiral determination, it too relies upon fitting scattering data and involves human handling, and it is not absolutely failsafe.

Case study #3: unanticipated effect of substituents in NMDA SAR.

N-Methyl-D-aspartic acid (NMDA) antagonists have received a resurgence of attention, particularly as compounds are tested in clinical trials following the discovery that ketamine can have fast-acting antidepressant effects.[26] One example of an NMDA antagonist is AstraZeneca's AZD6765 which has an established *S* absolute stereochemistry determined by X-ray crystallography of the dihydrochloride salt. The *S* enantiomer of AZD6765 binds more strongly to the target than the *R* enantiomer.

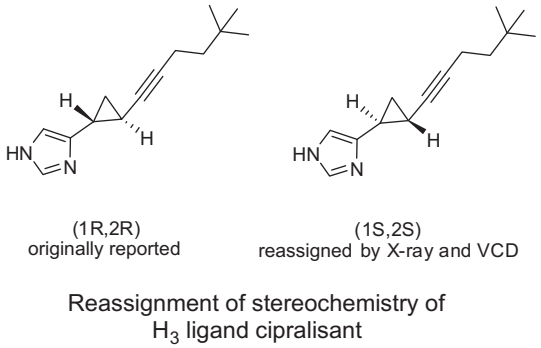
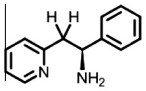


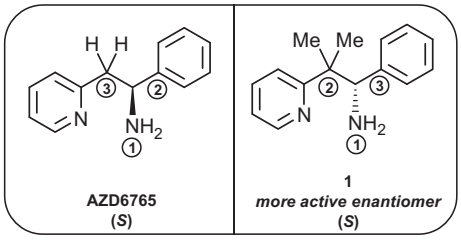
Figure 6. Structure of the H_3 ligand cipralisant. The stereochemistry was reassigned based on separate X-ray analyses at Abbott (Ref. 24) and VCD analysis at GSK (Ref. 25).



AZD6765
(S)

Our VCD assignment of the *gem*-dimethyl substituted compound 1 shows unambiguously that the stereochemistry of the enantiomer with greater affinity to the target is not the same as that of AZD6765 (NH_2 into the plane versus out of the plane, see Figs 7 and 8). The experimental VCD spectrum of 1 (purple spectrum) matches the simulated VCD spectrum with the NH_2 group pointed into the plane as drawn (green spectrum) and is opposite that of the simulated VCD spectrum with the NH_2 group out of the plane (red spectrum).

During lead optimization, it is quite useful to have rapid absolute stereochemical assignment, particularly if one enantiomer is not consistently the more active enantiomer within a series. In such cases, clear assignment of the absolute stereochemistry provides direct interpretation of SAR and avoids assumptions about the biological activity associated with each enantiomer. It also eliminates the ambiguity of using elution order from chiral chromatography (or other empirical observations) as a surrogate for tentative chiral assignment and SAR interpretation. There is no heavy atom in the ligand (i.e., a sulfur atom or larger), and standard X-ray crystallography is reliant upon use of a heavy-atom salt, although more sophisticated analyses are possible without this requirement.[27] VCD assignments, however, do not require crystals at all and could be performed shortly after synthesis of compounds in this series. In this case, the unanticipated stereochemical finding for 1 was determined by VCD and later confirmed by crystallographic studies on salts. Interestingly, nomenclature rules dictate



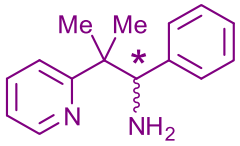
AZD6765
(S)

Figure 8. Structures of AZD6765 and 1. While the compounds have 'opposite' stereochemistry, Cahn-Ingold-Prelog priority rules dictate that both compounds are named with the *S* designation.

that the more active enantiomer for both AZD6765 and 1 are named with the *S* designation even though they have the 'opposite' stereochemistry (Fig. 8).

Additional examples. VCD assignments are becoming part of the organic and medicinal chemistry mainstream as evidenced by the growing number of examples in the literature. Figure 9 provides only a sampling of additional examples that demonstrate the types of compounds and applications that are possible (e.g., assignment of GABA modulators that exhibit atropisomers with axial chirality).[28–34]

Advances in VCD instrumentation and software. We have written an extensive in-house program under a single platform that serves as the launch point for unattended sample acquisition and generation of simulated spectra as well as the conduit to visualization and interpretation tools. This has been critical for the efficient (essentially unattended) acquisition and simulation of spectra. As important, the instrumentation and detection advances (even within a two year span) are exemplified in Figure 10. A series of mGluR2 positive allosteric modulators that required 25 mgs of sample to achieve sufficient signal for chiral assignment in our first efforts in VCD needed only 2 mgs to achieve comparable signal using a modified system. Reducing the sample requirement to a few milli-



1
more active enantiomer

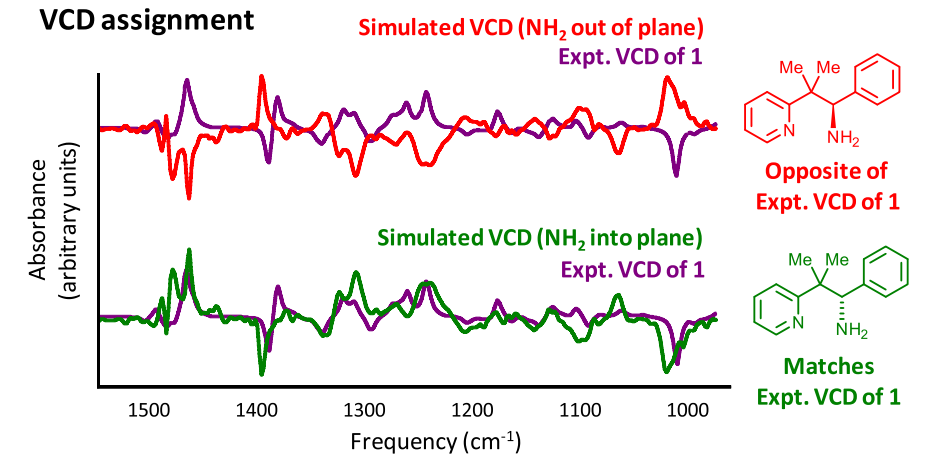


Figure 7. Chiral assignment of the *gem*-dimethyl compound 1 (more active enantiomer). The experimental VCD spectrum of 1 (purple spectrum) matches the simulated VCD spectrum with the NH_2 group pointed into the plane as drawn (green spectrum) and is opposite that of the simulated VCD spectrum with the NH_2 group out of the plane (red spectrum). Note the comparison of absolute stereochemistry assignment to AZD6765 which has the NH_2 pointed out of the plane as drawn (see Fig. 8). The simulations used computed spectra at the 6-311++G**/B3PW91 level of theory.

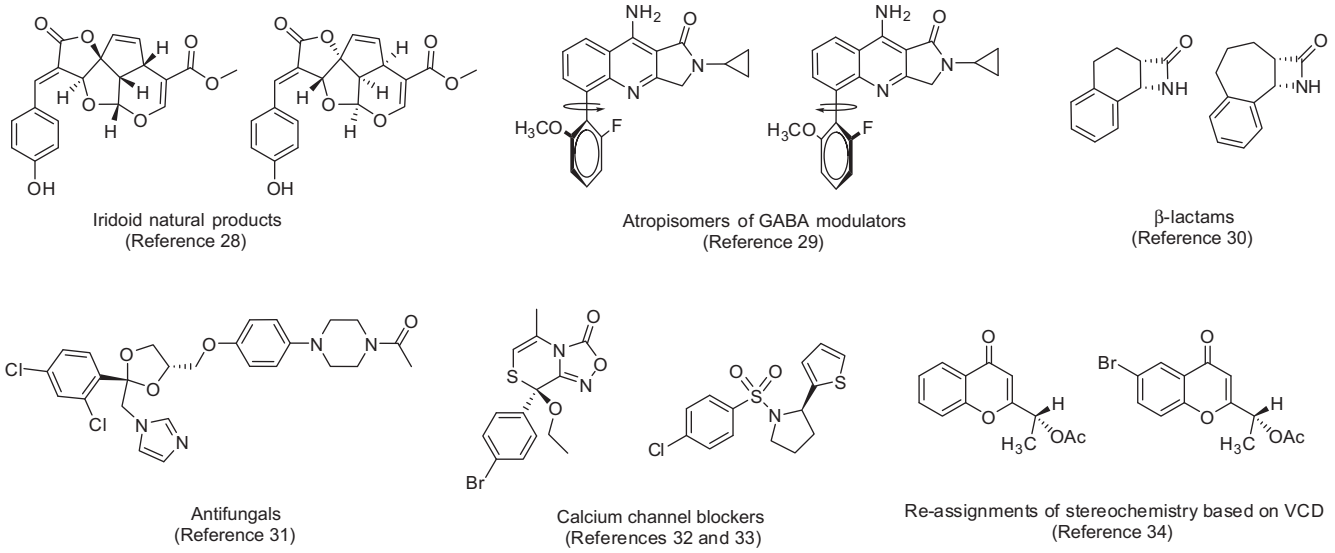


Figure 9. Additional examples of VCD assignments spanning several therapeutic areas. Examples of atropisomer assignment as well as examples of stereochemistry re-assignment based on VCD analyses are included.

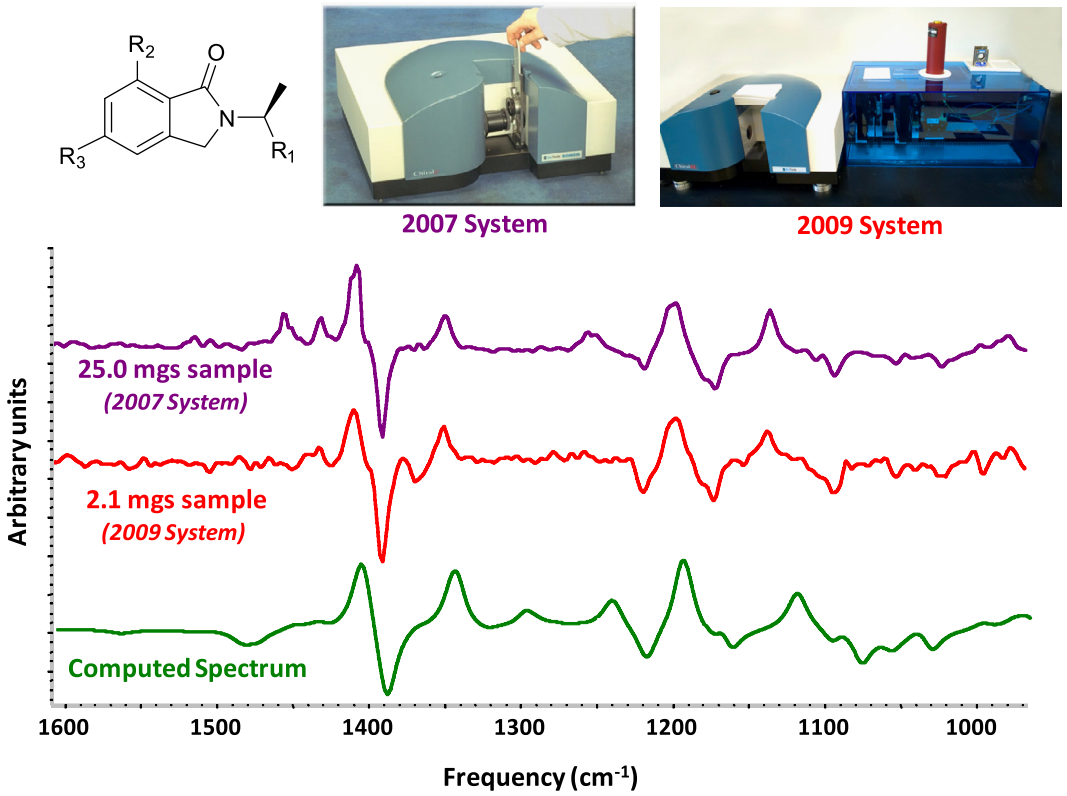


Figure 10. The practical impact of advances in VCD hardware. The entry-level technology in 2007 could require as much as 25 mgs of sample to achieve suitable signal-to-noise ratios. Incorporation of customized robotics, 48 h detector and rebuilt signal amplifiers provides significant enhancement of sensitivity and facilitates unattended sampling. In this chemical series, the sample requirement was reduced 10-fold to ca. 2 mgs while retaining sufficient signal for matching the computed spectrum.

grams is a key attribute for any realistic hope for high-throughput usage within an active discovery program where first-synthesized batches may be of a limited quantity and have biological and ADME screens as a higher priority.

Likewise, quantum chemistry packages have become faster and more robust.[35,36] While more rigorous QM methods (e.g., coupled-cluster theory) are still too computationally intensive for most drug-like compounds, density functional theory (DFT) has been remarkably accurate for chiral assignment.[7] Furthermore, molecules even larger and more flexible than those shown in Figure 9 may often be assigned by measuring the VCD spectrum of the full molecule, but matching to a truncated model system for the DFT simulations where parts of the molecule far from the chiral center (and thus are unlikely to contribute significantly to the VCD signal) are deleted. In addition to computational speed, developments in commercial software over the next decade will likely include

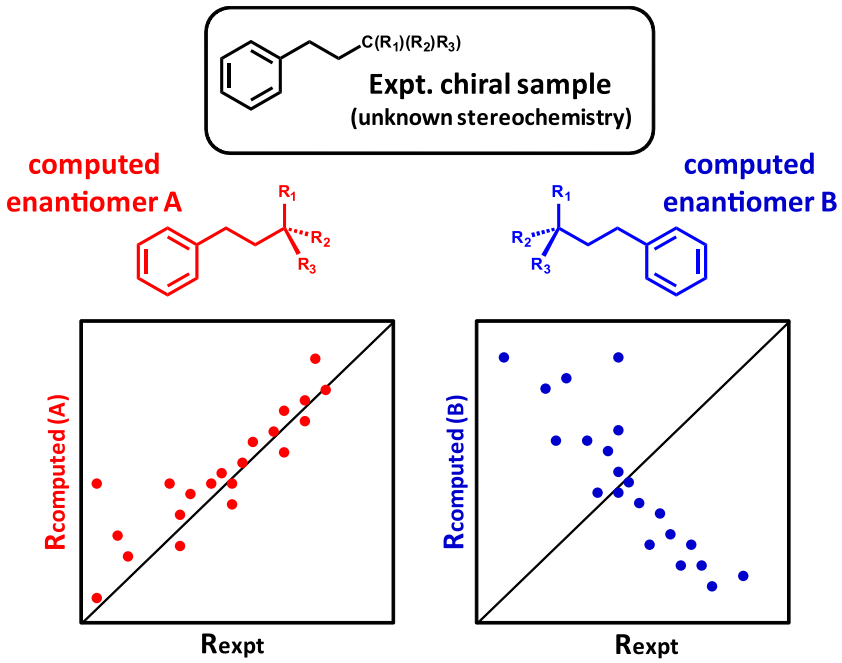


Figure 11. Schematic of the correlation analysis of the measured rotational strengths (proportional to VCD intensity) and the Boltzmann-weighted computed rotational strengths for each enantiomer advanced by Stephens et al. (for example in Ref. 28). The match of the experimental rotational strengths is clearly better for computed enantiomer A (red) than for computed enantiomer B (blue). Such quantitative analyses supplement visual matching of measured and simulated VCD spectra.

automation or guided navigation of steps like Boltzmann weighting and line broadening of the composite simulated spectra such that many aspects of in-house software may be superseded.

Metrics of fit and confidence level of assignment. Since VCD chiral assignments rely upon comparisons to simulated spectra, an assessment of the quality of the fit is essential. This is an active area of research among both academics and commercial vendors. Perhaps the most straightforward approach is that of Stephens who correlated the measured and computed rotational strengths (proportional to VCD intensity) for the spectra as illustrated in Figure 11.[28] More sophisticated mathematical fits of the measured and simulated fingerprint region are under investigation and will likely continue to produce additional metrics of 'goodness of fit' and thus a quantitative confidence level of assignment. Kuppens et al. introduced the notion of using overlap integrals as the basis for a similarity index[37] and further advances in VCD neighborhood similarity and enantiomeric similarity indices have been published[38] and implemented in commercial software (e.g., *CompareVOA*™).[39] Such metrics may be converted into a confidence level of correct prediction and may be compared to databases of compounds with known absolute stereochemistry. The field is moving toward the development of a VCD analog to the Flack parameter commonly used in X-ray crystallography.[40] In our experience, spectra lacking peak-to-peak matching should be further interrogated (e.g., assessment of sample purity, potential for hydrogen-bonded complexes, completeness of basis sets and DFT functional employed in the simulations, etc.). While not all assignments have been subsequently validated using X-ray analysis or stereo-specific synthesis, all samples for which complementary data have become available have been consistent with our VCD assignments. Any deviation from such a track record would naturally erode the confidence chemists would have in this technique.

Accessibility and acceptance. As pharmaceutical companies become more nimble and venture into partnerships with third parties, VCD has also become accessible via relationships with universities and companies with expertise in this area. For example, BioTools, Inc. and other companies offer fee-for-service chiral assignment that may be a practical option for pharmaceutical companies lacking in-house instrumentation or expertise. Particularly as candidate drugs are advanced, confirmation of absolute stereochemistry using a complementary technique is a sound investment. Furthermore, the FDA has now recognized VCD as an acceptable method for assignment of absolute stereochemistry.

As recently as 5 years ago, the inherent complexity of the VCD experiment and the quantum mechanics simulations placed this technology only in the hands of relatively few academics and industrial groups that happened to enjoy significant expertise in both experimental vibrational spectroscopy and theoretical quantum chemistry. X-ray crystallography, optical rotation, and electronic circular dichroism all will continue to serve as important and complementary techniques for assignment of absolute stereochemistry—indeed, the confidence of the assignment increases when independent techniques converge on the same conclusion. The wealth and density of peaks generally present in a VCD spectrum matched with high-level simulations provides an elegant means of assigning stereochemistry with high fidelity. With increasingly vibrant interactions between industry and academia as well as close ties to vendors of VCD instrumentation and quantum chemistry software packages, this technology is rapidly coming of age as a mainstream tool in the arsenal of pharmaceutical research.

Acknowledgments

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